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THE EFFICACY OF THE AQUEOUS EXTRACT OF *ADANSONIA DIGITATA* AGAINST *PLASMODIUM BERGHEI* INFECTION IN MICE

***[1]Danlami Elisha Akafyi, [1]Rashida Dewu Abba, [2]Isa Danladi Jatau, [1]Amaya Jobin Habila and [1]Folashade Serah Ojeleye**

[1]Department of Science Laboratory Technology, Nigerian Institute of Leather and Science Technology, Zaria, Nigeria.
[2]Department of Vertenary Parasitology, Ahmadu Bello University Zaria, Nigeria

*Corresponding authors' email: dakafyippp@gmail.com

ABSTRACT

The prevalence of malaria remains a significant threat in Sub-Saharan Africa. The occurrence of drug-resistant strains of Plasmodium in Nigeria underscores the urgent need for novel antimalarial medications. This research aims to assess the effectiveness of *Adansonia digitata* fruit pod extract against Plasmodium berghei infection in mice; determining the oral median lethal dose (LD50) and identifying phytochemical components using Lorke's and Evan's methodologies. The extract was assessed for its antiplasmodial activities against *P. berghei-berghei* in two murine malaria replicas using both suppressive and curative assays. Statistical analysis was conducted using one-way ANOVA. The LD50 of the extract through oral administration exceeded 5000 mg/kg. The extract contains alkaloids, saponins, flavonoids, phenols, tannins, and anthraquinones. The aqueous fruit pulp extract of *A. digitata*, when administered at 250, 500, and 1000 mg/kg of doses, exhibited a dose-dependent suppression of parasitemia by 25.70%, 42.57%, and 49.39% respectively compared to the control group, parasitaemia clearance rates was 31.76%, 43.34%, and 56.65% respectively. The parasitaemia clearance with a dosage of 1000 mg/kg was found to be statistically significant ($p < 0.05$).The reference drug (Chloroquine) administering a dosage of 5 mg/kg yielded a significant result ($p < 0.05$) parasitaemia clearance of 74.76%. Analysis of the Packed Cell Volume (PCV) levels revealed that those treated with the aqueous fruit pulp extracts of *A. digitata* at doses of 250, 500, and 1000 mg/kg maintained normal PCV values throughout suppressive and curative experiments. However, the PCV in the control groups was markedly diminished ($p < 0.05$) after both trials.

Keywords: Malaria, *P. berghei-berghei*, Extract, Parasitemia, *Adansonia digitata*

INTRODUCTION

Malaria is a significant health issue that affects a large population in Africa. It is caused high mortality (Bantie et al., 2014). Malaria is common in tropical and subtropical regions and is often associated with poverty. Its symptoms include headache, fever, chills, and vomiting. If not treated promptly, it can lead to serious complications (WHO, 2018).

The parasite is becoming more resistant to drugs and the insects that spread the disease are also becoming resistant to insecticides. In addition, the lack of infrastructure in countries where the disease is common is making it difficult to control. Because of these challenges, it is crucial to find new drugs that can effectively treat the disease. Current antimalarials are also facing resistance globally (Saurabh and Richa 2024). The demand for new drugs is urgent and critical. We need different treatment methods that can effectively treat all strains of the disease, including those that are resistant to current drugs. These treatments should also be able to clear the parasite from the body to achieve better treatment results.

Artemisinin (ART), the preferred pharmacological agent for malaria prophylaxis, is synthesized from natural botanical sources and its derivate are used in Artemisin-based combination therapy (ACT) has helped the fight against malaria (Bamalli*et al*. 2006). Plants provide a rich source of drugs for anti-malaria as argued by Hilou *et al*. (2006). From ethnomedicine data, it is documented that many plants are used in control and treatment of malaria however, very few of these plants have been analyzed chemically (Garavito*etal*., 2006).*A. digitata*, is a plant that belongs to the Bombacaceae family. Its leaves and fruits are used in Orthodox medicine and contain alkaloids, saponins, flavonoids, phenols, tannins, and anthraquinones. (Kamatou*et al*., 2003).

A. digitata is a common plant in Nigeria with significant medical value. Research on its antimalarial properties aims to identify compounds with high antimalarial potential and validate its use in folklore medicine. The research investigates the efficacy of the plant extract on *P*. berghei infection in the experimental animals, providing essential data on its potential as a safe antimalarial compound.

MATERIALS AND METHODS

Plant material

The fruit pods of *A. digitata* were collected on farms in Samaru, Zaria. (Coordinate 11.1247°N, 7.7254°) The pods were taken to the Herbarium Unit at Ahmadu Bello University for identification and authentication and assigned voucher number ABU02512. The seeds were pounded to remove the fruit pulp, separated from the seeds and fibers. The product were stored at room temperature for analysis.

Formulation of *A. digitata*

One hundred grams (100g) of the powdered fruit sample was placed into a two-litre volume plastic container; 1.5 litre of distilled water was added to the content, tightly screw-capped and shaken thoroughly. The container was shaken thoroughly twice at 2-hour intervals (Talukdar, 2010). After 24 hours, the contents were gently streamed using a mesh material into a clean container. Residues were re-soaked in distilled water in the same container and allowed to settle for another 24 hours and shaken as done previously then filtered in the container where the initial filtrate was collected and the residue discarded. The filtrate was placed in a glass container in a water bath to obtain the extract's solid and concentrated form.

Phytochemical study

The sample of *A. digitata* were studied for phytochemicals using the methods described by Talukdar (2010). To test for carbohydrates, 500 mg of each extract was dispensed to a test tube, 3 drops of Molisch reagent and 1 mL of sulphuric acid were added. Carbohydrate presence identify by reddish colour. A Half grams of the extract was dissolved in a ferric chloride medium to test for glycosides. The emergency of a purple-brown ring is indicative for the presence of sugars. Tannins test was carried out by placing 1millimetre of the extract and ferric chloride into a test tube and observed for a greenish-black precipitate indicating tannins' presence and a blue or brownish-blue precipitate for hydrolyzable tannins. To ascertain the presence of saponins, an aliquot of 10 millilitres of distilled water was incorporated into the extract, which was then subjected to agitation for a duration of 30 seconds within the test tube, followed by a period of observation lasting 30 minutes. The presence of saponins is confirmed when the frothy emulsion remains stable for a duration of 10 to 15 minutes.Flavonoids were tested in the tube by adding 5 drops of 10% sodium hydroxide into 200mg of the extract. A yellow colouration indicates flavonoids.To test for alkaloids, 200 mg of the extract and 5 drops of Dragendorff's were dispensed to a tube for a reddish-brown precipitate, which indicates alkaloids.

100 mg of the extract was mixed with 5 mL of chloroform to test for Anthracene. The admixture was agitated for a duration of five minutes and subsequently subjected to filtration. Thereafter, an equivalent 10% ammonia solution was incorporated into the filtered substance. The test tube was scrutinized for the presence of a vivid pink hue in the aqueous layer, which serves as an indicator of anthraquinones.

Experimental Animals

The experiment involved 62 adult mice, each weighing between 20-25g and housed in a pen within a well-netted animal house. The mice were acclimatized for two weeks and fed with commercial chick grower mesh and provided with water ad libitum.

Inoculum

Plasmodium berghei was obtained from two infected donor mice in Ahmadu Bello University, Zaria. The blood was collected in a beaker, containing EDTA. 2ml of normal saline was added into the beaker and shaken gently.

Lethal dose test

The median lethal dose (LD50) of the *A. digitata* extract were assessed using Lorke's (1993) method. Initially, three groups of three mice received oral doses of the extract at 10^1, 10^2, and 10^3 mg/kg, and were monitored for 24 hours for toxicity or mortality. In a second phase, single mice in three groups were given doses of 1600, 2900, and 5000 mg/kg, also observed for 24 hours. The LD50 was calculated as the geometric mean of the highest non-lethal dose and the lowest lethal dose.

Experimental Design

Fifty (50) adult male and female albino mice (*Mus musculus*) of 20-25g average in weight was used. The mice were chosen and separated into two (2) categories of 25 mice respectively, for the suppressive and curative tests respectively. Mice in each tests group were divide into 5 subgroups of 5 mice each in Group I, negative control (Infected with *Plasmodium berghei*, Group II, positive control (Infected and treated with chloroquine as standard drug);Groups III, IV and V (infected and treated with 250, 500 and 1000mg/kg of the test extract respectively). All mice were infected with 0.25 mL of blood of 1×10^7 *P. berghei*-infected blood and were treated 3 and 72 hours later for the tests groups respectively.

***In-vivo* Antimalarial Activity**

To evaluate the extracts against early infection in mice. A 4-day standard test as carried out by Peter *et al.* 1995. Mice were inoculated by intraperitoneal injection with 1×10^7 *P. Berghei-infected* blood. After 3 hours, treatment was administered orally once daily for 4 consecutive days with 100, 250, and 500 mg/kg of all the extracts orally. 10 mg/kg chlorine was administered daily to the positive control mouse, the negative control group was infected and given normal saline, and the neutral control was neither infected nor treated. Following the treatment of the final dosage (D4), a blood specimen was procured from the tail of each murine subject, subsequently a thin smear was generated, preserved in absolute methanol, stained utilizing Giemsa solution, and analyzed under a microscope at a magnification of × 100. Parasites were counted to assess the parasitaemia in ten random fields of blood smear samples from each mouse to determine the mean parasitaemia level for each group.

The Potency ability

The test for potency was used to evaluate the extracts in established infection. This was done as described by Fraga (1988). Mice from various groups (n=5) were inoculated by intraperitoneal injection on day one (Do). 72 hours later (D3), the extract (250, 500, 1000 body weight (mg/kg), groups II-IV) were administered to the mice. The standard drug and deionized water were administered to the experimental and control groups, respectively. Treatments were administered daily for five days. Pre-treatment (D3) and Post-treatment (D7) parasite levels were determined by stained thin smears from tail blood samples collected on each day respectively, as described above.

Packed Cell Volume (PCV) determination

The PCV of the experimental mice were determine before and after the study to assess the extract's effectiveness in preventing hemolysis caused by increased parasitemia. Blood was collected using the pricking method and then dropped into a capillary tube, filled to a marked point, a residual 15 mm remained unoccupied. The tubes were subsequently sealed through a process of flaming one end and subsequently spun at 15000 r.p.m for 5 minutes. Packed Cell Volume was then measured using a Hawksley microhaematocrit reader (Gellman Hawksley Ltd, 92 England) as described by Simo *et al.*, (2006).

Data analysis

The results were expressed as the mean ± standard error of the mean (SEM). They were analyzed using One-way analysis of variance (ANOVA). Results were considered at $p \leq 0.05$ level using SPSS software version 20.

RESULTS AND DISCUSSION

Six constituents of the phytochemical of *A. digitata* result

Table 1: Constituents of Aqueous Extract of *Adansonia digitata*

.	Phytochemical constituents	Result
	Phenol	+
	Tannins	+
	Saponins	+
	Flavonoid	+
	Cardiac glycoside	-
	Terpenoids	-
	Steroids	-
	Alkaloids	+
	Anthraquinones	+

+ = Present, - = Absent

The Chemo-suppressive antiplasmodial test of *A.digitata* at the 250 mg/kg doses were administered x1, x2 and x4 produced a dose-dependent parasitaemia suppression of 25.70, 42.57, and 49.39% respectively. The control drug (Chloroquine) at 5 mg/kg showed significant parasite reduction of 78.55% (Table 2). The test for cure of the extract of *A. digitata* 250 mg/kg doses were administered x1, x2 and x4 produced dose-dependent parasitaemia clearance of 31.76%, 43.34% and 56.65% respectively.

Table 2: The Chemo-suppressive suppressive influence of the extract on murine subjects infected with *P. berghei.*

Treatment (mg/kg)	Parasitaemia Suppression	Chemo suppression(%)
Negative control	24.9 ± 1.14^{a}	-------
Positive control	5.34 ± 0.62^{c}	78.55
250mg/kg	18.5 ± 0.45^{b}	25.70
500mg/kg	14.3 ± 0.53^{ab}	42.57
1000mg/kg	12.6 ± 0.19^{ab}	49.39

Data is presented as mean ± SEM, n=5.
Means with superscripts showed association at $p<0.05$.

Table 3: The therapeutic efficacy of the extract on mice infected with *P. berghei*.

Treatment (mg/kg)	Parasitaemia D3	Parasitaemia D7	Parasite Clearance (%)
Negative control	21.1 ± 0.52^{a}	23.3 ± 0.29^{a}	------
Positive control	22.1 ± 0.33^{a}	5.88 ± 0.51^{b}	74.76
250mg/kg	22.1 ± 0.45^{a}	15.9 ± 0.63^{ab}	31.76
500mg/kg	22.0 ± 0.53^{a}	13.2 ± 0.43^{ab}	43.34
1000mg/kg	22.4 ± 0.54^{a}	10.1 ± 0.85^{b}	56.65

Data is presented as mean ± SEM, n=5.
Means with superscripts showed association at $p<0.05$.

Table 4: Chemo-suppressive effect on the PVC of *P. berghei-infected* mice

Treatment (mg/kg)	PCV Pre- infection (%)	PCV Post-treatment (%)
Negative control	29.8 ± 0.583^{a}	24.0 ± 0.548^{b}
Positive control	31.0 ± 0.707^{a}	31.0 ± 0.837^{a}
250mg/kg	30.8 ± 0.735^{a}	28.6 ± 0.678^{ab}
500mg/kg	30.2 ± 0.374^{a}	29.4 ± 0.245^{a}
1000mg/kg	28.8 ± 0.53^{a}	28.0 ± 0.316^{ab}

Data is presented as mean ± SEM, n=5.
Means with superscripts showed association at $p<0.05$.

Table 5: The therapeutic efficacy of the extract on the PVC of *P. berghei-infected* mice

Treatment (mg/kg)	PCV Pre-infection (%)	PCV Pre-treatment (%)	PCV Post-treatment (%)
Negative control	31.4 ± 1.12^{a}	23.8 ± 0.917^{b}	19.6 ± 0.678^{b}
Positive control	30.4 ± 0.51^{a}	29.0 ± 0.316^{a}	32.0 ± 0.548^{a}
250mg/kg	29.8 ± 0.66^{a}	26.8 ± 0.74^{ab}	26.6 ± 0.4^{ab}
500mg/kg	33.2 ± 1.16^{a}	28.6 ± 2.67^{ab}	27.4 ± 0.68^{ab}
1000mg/kg	30.2 ± 1.32^{a}	27.4 ± 0.927^{ab}	24.6 ± 0.41^{ab}

Data is presented as mean ± SEM, n=5.
Means with superscripts showed association at $p<0.05$.

Discussion

The current investigation elucidated that *A. digitata* is imbued with chemical constitutes, including saponins, flavonoids, alkaloids, and anthraquinones, which are responsible for numerous pharmacological activities related to the treatment of certain diseases. This study showed similar of reports of Anani *et al*. (2000) and who also identified similar phytochemicals of *A. digitata*.

Quinine, known as the most ancient antimalarial pharmaceutical agent, is an alkaloid extracted from the

cinchona tree's bark (Biamonte et al., 2013). Furthermore, Rasoanaivo *et al*. (2011) and Kaur et al. (2009) have demonstrated that flavonoids showed antiplasmodial properties by inhibiting fatty acid biosynthesis in parasites, consequently disrupting metabolic processes and resulting in death. Hence, these phytochemicals present may be attributed to the observed antiplasmodial effect in this study. These phytochemicals action may be in single constituents or in combination for the seen effect against the parasite.

The findings of the test showed that it is safe to administer aqueous *A. digitata* at 9000 mg/kg which showed similar result with the report of Ogunleye *et al*. (2019) whose finding showed that pulp is safe at 9000 mg/kg. As such, it is a good alternative antimalarial agent.

The results of PCV analysis show that there was no effect in PCV values in the experimental animal before and after the study and the standard drug in both the suppressive and the therapeutic tests, this suggests that the plant was effective in maintaining the PCV levels as the extracts prevented lysing of the RBCs by the plasmodium parasites which is evident in rodent malaria. Mice malaria produces parasitemia reduction in PCV at approximately 48hrs post-infection (Bantie*et al.*, 2014) due to anaemia resulting from red blood cells rapture, due to the *p. berghei* multiplication (Dua, 2013).

CONCLUSION

The study revealed that the aqueous extract of *A. digitata* is non-toxic and contains active phytochemicals such as flavonoids and alkaloids, which have the potential for in vivo antiplasmodial effects. It also showed associted antiplasmodial activity in curative rodent malaria models, particularly at the tested dose of 1000mg/kg.

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